Evaluation of Event Related Potentials in Vitamin B12 Deficient Patients with Functional Cognitive Deterioration

Vitamin B12 Eksikliğine Bağlı Kognitif Fonksiyon Bozukluğunda Olaya İlişkin Potansiyeller

Vitamin B12 ve Olaya İlişkin Potansiyeller / Vitamin B12 and Event Related Potentials

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Özet

Amaç: Bu çalışmada, vitamin B-12 replasman tedavisinin, vitamin B-12 eksikliği olan hastaların kognitif fonksiyonları ve olayla ilişkili potansiyeller üzerindeki etkilerinin değerlendirilmesi amaçlanmıştır. Gereç ve Yöntem: Bireylerin serum vitamin B-12 düzeylerine göre 3 grup oluşturuldu.Vitamin B-12 düzeyi ≤197 pg/ml ve kognitif yakınmaları bulunan 16 hasta grup 1'de yer aldı. Grup 2 (n=17, vitamin B-12 seviyeleri 198-350 pg/ml arası) ve grup 3 (n=16, vitamin B-12 seviyeleri 350 pg/ml'nin üzerinde) sağlıklı bireyler tarafından oluşturuldu. Tüm bireylere standardize mini mental durum testi (SMMDT) uygulandı. Grup 1' deki hastalara, SMMDT tedaviden 3 ay sonra tekrar uygulandı. B-12 replasman tedavisinin etkinliğini değerlendirmek amacıyla, grup 1' de olayla ilişkili potansiyeller tedavi öncesi ve sonrası değerlendirildi. Bulgular: Grup 1' de, tedaviden sonraki P300, N200 ve P200 amplitüdleri artmış ve P300, N200 and P200 latansları ise kısalmış bulundu. Tartışma: Bu çalışmanın sonuçları, P300, N200 ve P200 amplitüd ve latansları ile birlikte SMMDT skorlarındaki düzelmenin vitamin B-12 replasman tedavisinin etkinliğinin değerlendirilmesinde kullanışlı parametreler olabileceğini göstermiştir.

Anahtar Kelimeler

Olaya İlişkin Potansiyeller; Kognitif Fonksiyonlar; Vitamin B12; P300; N200

Abstract

Aim: This study was conducted to evaluate the influence of vitamin B-12 replacement therapy on event-related potentials (ERPs) and cognitive functions of the patients with vitamin B-12 deficiency. Material and Method: Patients were divided into three groups according to the serum vitamin B-12 levels of individuals. Sixteen patients with serum vitamin B-12 levels ≤197 pg/ml and with complaints of cognitive dysfunction were enrolled in the group 1. Group 2 (n=17, with serum vitamin B-12 levels between 198-350 pg/ml) and group 3 (n=16, with serum vitamin B-12 levels above 350 pg/ml) were formed by healthy individuals. Standardized mini mental state examination (SMMSE) test was performed to all individuals. Also the SMMSE test was applied to group 1 again 3 months after the treatment. In group 1, to measure the effectiveness of vitamin B-12 replacement therapy, ERP studies were performed before and after treatment. Results: In group 1, P300, N200 and P200 amplitudes were found to be increased, and P300, N200 and P200 latencies were found to be shortened after the treatment. Discussion: Study results suggest that improvement in P300, N200 and P200 amplitudes and latencies together with SMMSE scores are useful parameters in the assessment of vitamin B-12 replacement therapy.

Keywords

Event-Related Potential; Cognitive Functions; Vitamin B12; P300; N200

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Introduction

Vitamin B-12 deficiency is common especially in older adults, and its prevalence increases with age. The clinical manifestations of vitamin B-12 deficiency are both hematological and neurological. The neurological consequences differ in a wide range that includes peripheral neuropathy, subacute combined degeneration of the spinal cord, cognitive impairment, dementia and depression. Some studies revealed the association between the elevated total homocysteine concentrations and cognitive impairment [1-3]. However, the role of low vitamin B-12 status in cognitive decline still remains unclear.

Event-related potentials (ERPs) is a widely used non-invasive method for manifesting the brain activity during cognitive processes. Previous studies demonstrated that the P300 amplitude is related to the updating of working memory content, and the P300 latency is related to the speed of stimulus evaluation [4-6]. Abnormally prolonged P300 latencies have been reported in Parkinson's disease, Alzheimer's disease, dementias and depression [7-9]). A recent case report showed that the cognitive improvement after vitamin B-12 replacement is associated with a significant improvement of the P300 latency [10].

The aim of this current study was to assess the efficacy of B-12 replacement therapy in patients with cognitive decline due to vitamin B-12 deficiency by using the ERPs parameters.

Material and Method

Subjects

Patients, aged from 20 to 60 years, who has subjective cognitive complaints (short term memory loss, attention deficiency, learning difficulty etc.) for the last 3 months were screened for vitamin B-12 deficiency to adjust the study groups. Three groups were arranged according to serum vitamin B-12 levels. Sixteen patients (13 female and 3 male) whose serum vitamin B-12 levels were below 197 pg/ml were enrolled in the Group 1. Goups 2 and 3 were taken from healthy volunteers without any complaint. Group 2 constituted of 17 subjects (14 female and 3 male) whose serum vitamin B-12 levels were between 198 and 350 pg/ml., and group 3 constituted of 16 subjects (12 female and 4 male) whose serum vitamin B-12 levels were above 350 pg/ml. Our study was approved by Local Ethics Committee and informed consent forms were obtained from all participants.

Patients who are vegetarian, pregnant, who had a history of central nervous system (CNS) infection, tumor involving CNS, head trauma, any intracranial operation, stroke, diabetes mellitus, gastrointestinal surgery, atrophic gastritis, malabsorbtion, thyroid dysfunction, anemia, any psychiatric disease and who use drugs effecting CNS were excluded from the study.

Procedure

All participants evaluated by a cardiology consultant, systemic and neurological examinations, complete blood countings, sedimentation rates, hepatic and renal function tests, serum electrolytes, lipid profiles and serum thyroid hormone levels were checked for all groups. Education levels of the subjects were also recorded.

We assessed global cognitive function, including evaluation of memory, orientation (to time and to place), attention, calculation, language and repetition by using the Standardized Mini Mental State Examination (SMMSE) for all participants. Patients in group 1 were assigned to receive vitamin B-12, 1 mg/day per orally for 2 months and SMMSE test repeated 3 months after the treatment in order to evaluate the efficiacy of the vitamin B-12 replacement therapy.

All participants underwent ERP studies, additionally, ERPs were recorded in group - 1 three months after treatment in order to assess the efficiacy of vitamin B-12 replacement therapy. Group 2 and 3 did not need control ERP study because basal electrophysiologic values were normal(Table2) and they were not given B12 replacement therapy. So group 2 and 3 did not undergo ERP study.

Four-channel phases apparatus (Esa-Ote Biomedica, Florence, Italy) used for the study. ERPs were recorded (filtered bandpass: 0,1-50 Hz, analysis time: 1 sec) by using scalp AgCl electrodes 11 mm in diameter. Interelectrode impedance was maintained at less than 5kΩ. Electrodes were placed according to the international 10 - 20 system and recordings were performed at sites Pz and Fz. Ground electrode was placed at Fpz. Channel-1 was placed at infraorbital (IO) site to eliminate the artifacts caused by eyelid movements. A standard ''oddball'' paradigm was used to elicit the auditory P300. A series of binaural tones at 60 dB sound pressure level with a 10 ms rise/fall and a 100 ms plateau time was presented to all subjects. The auditory stimuli were presented in a random sequence with target tones of 2000 Hz occurring 20% of the time and standard tones of 1000 Hz occurring 80% of the time at a rate of 0.5 Hz. Each subject was asked to count only the target tones and report the total number at the end of the test. 40 artifact-free target tones were averaged and each test was performed twice to ensure that waveform components are reproducible.

Statistical Analysis

The peak latencies and amplitudes of P300, N200 and P200 were measured for all subjects. Statistical analysis was performed with SPSS 15.0 for Windows (SPSS Inc). Of the measured parameters, sex and education level were analyzed by Chi-square test. ANOVA test was used to determine differences in age, ERP and SMMSE parameters between three groups. Probability values <0.05 were considered statistically significant. Paired t-test was used to compare the SMMSE scores of group 1 before and after treatment.

Results

Systemic and neurological examinations of the subjects were all normal. No anemia was detected in any of the subjects. The mean serum vitamin B-12 level in group 1 which was measured 3 months after the B-12 replacement therapy was 283±12 pg/ml (range between 228-327 pg/ml).

Statistically, no significant differences were found in age between the groups (mean age for groups 1,2,3 was 42.3±12.6, 45.2±13.9 and 39.3±10.8 respectively; p=0.408). There was no significant differences in the education levels and sex characters between the groups (p>0.05).

SMMSE scores of groups 1, 2, 3 was 26.63, 28.14 and 27.38 respectively, before the treatment ANOVA test revealed significant differences in SMMSE scores between group 1 and group 3 (p=0.04) ,but no difference between group1 and group 2

(p=0.181), and between group 2 and group 3 (p=0.773). In group 1, SMMSE scores was found to be statistically improved after vitamin B-12 replacement therapy (p<0.05) (Table1).

ERP parameters and statistical analysis results seen at table 2. When compared to group 1, group 2 and 3 revealed statistically significant difference in ERP results before the treatment. After B12 replacement these differences disappeared .

All subjects responded the target tones of the ERPs with an accuracy of over 95%. In group 1 P300, N200 and P200 amplitudes were found to be increased, and P300, N200 and P200 latencies were found to be shortened after the treatment. Results were statistically significant (p<0.05) (Table2).

Table 1. SMMSE scores and statistical analyses of SMMSE score comparisions between groups and before and after B12 replacement therapy.

		SMMSE score Mean ± SD	Group 1 BT vs. Group 1 AT P	Group 1 BT vs. Group 2 P	Group 1 BT vs. Group 3 P	Group 2 vs Group 3 P	Group 1 AT vs. Group 2 P	Group 1 AT vs. Group 3 P
Group 1	BT	26,63 ± 2,02	0.010	0.181	0.040	0.773	0.865	0.590
	AT	27,94 ± 1,06						
Group 2		28,14 ± 1,93						
Group 3		27,38 ± 1,21						

SMMSE: Standard Mini Mental Test Score, BT : Before B12 treatment, AT : After B12 treatment

Table 2. Frontal and parietal ERP records and statistical analysis of 3 groups before and after B12 treatment. Comparision of group 1 to group 2 and 3 showed statistically significant difference only before the B12 treatment.

	Group 1 Mean ± SD	Group 2 Mean ± SD	Group 3 Mean ± SD	Group 1 vs. Group 2 P value	Group 1 vs. Group 3 P value	Group 2 vs. Group 3 P value
PP3L						
Before treatment	346,88±31,42	321,68±42,55	318,71±28,18	0.023	0.014	0.195
After treatment	323,88±31,44			0.134	0.226	
PP3A						
Before treatment	13,17±9,71	17,83±8,15	19,05±7,43	0.034	0.026	0.367
After treatment	17,42±8,61			0.862	0.574	
PP2L						
Before treatment	170,02±15,29	169,42±12,93	166,93±15,29	0.036	0.207	0.031
After treatment	176,69±17,31			0.675	0.0529	
PP2A						
Before treatment	3,13±1,68	3,89±1,47	4,05±1,62	0.040	0.014	0.476
After treatment	3,93±1,58			0.493	0.552	
PN2L						
Before treatment	235,88±28,18	220,06±18,38	217,76±16,74	0.028	0.031	0.843
After treatment	220,94±19,47			0.094	0.107	
PN2A						
Before treatment	4,48±2,48	6,71±1,92	6,05±1,89	0.043	0.039	0.163
After treatment	6,11±2,00			0.145	0.367	
FP2L						
Before treatment	180,81±18,53	174,12±15,25	172,85±12,46	0.033	0.029	0.436
After treatment	175,38±13,83			0.098	0.122	
FP2A						
Before treatment	2,96±1,43	3,43±1,80	3,40±1,69	0.043	0.025	0.768
After treatment	3,45±1,73			0.368	0.456	
FN2L						
Before treatment	240,13±29,75	225,63±18,95	222,33±20,14	0.037	0.014	0.456
After treatment	227,19±22,86			0.148	0.099	
FN2A						
Before treatment	6,54±3,55	7,56±3,30	7,94±3,80	0.044	0.037	0.759
After treatment	7,38±4,40			0.175	0.344	
FP3L						
Before treatment	347,38±32,61	326,72±25,60	326,74±28,65	0.039	0.030	0.286
After treatment	328,19±32,45			0.528	0.483	
FP3A						
Before treatment	13,08±11,16	15,66±8,75	16,56±9,36	0.025	0.021	0.731
After treatment	15,94±11,08			0.328	0.239	

PP3L : parietal P300 latans, PP3A: parietal P300 amplitude, PP2L: parietal P200 latans, PP2A: parietal P200 amplitude, PN2L: parietal N200 latans, PN2A: parietal N200 amplitude, FP2L: frontal P200 latans, FP2A: frontal P200 amplitude, FP3A: frontal P300 amplitude, FP3L: frontal P300 latans, FN2A: frontal N200 amplitude, FN2L: frontal N200 latans

Discussion

The purpose of this study was to assess the influence of vitamin B-12 replacement therapy on ERPs in patients with impaired cognitive functions. Both vitamin B-12 and folate (vitamin B-9) are involved in a common metabolic pathway supplying essential methyl groups for DNA and protein synthesis. In particular, vitamin B-12 is the necessary coenzyme, adequate for the correct functioning of the methyl donation from 5 methyltetrahydrofolate in tetrahydrofolate, necessary for methionine synthetase. Folate is a cofactor in one-carbon metabolism, during which it promotes the remethylation of homocysteine-a cytotoxic sulfur-containing amino acid that can induce DNA strand breakage, oxidative stress and apoptosis [11-13]. In case of folate or vitamin B12 deficiency, the methionine synthetase reaction is severely impaired. Additionally, deficiency in either folate or vitamin B-12 leads to an increase in total serum homocysteine concentrations. However, as homocysteine concentrations are easily lowered by dietary supplementation with folic acid and vitamin B-12, we preferred this study to design based on the relationship between cognitive impairment due to low vitamin B-12 and ERPs parameters [12-15].

The diagnosis of vitamin B-12 deficiency has traditionally been based on low serum vitamin B-12 levels, usually less than 200 pg/ml (or <156 pmol/L), along with clinical evidence of disease [16,17]. Furthermore, measurements of metabolites such as methylmalonic acid and homocysteine have been shown to be more sensitive in the diagnosis of vitamin B-12 deficiency than the measurement of serum vitamin B-12 level alone [18-20]. However, it is also incontrovertible that measurement of serum vitamin B-12 level is currently used as a standard clinical screening test for vitamin B-12 deficiency.

Clinically, vitamin B-12 deficiency is known to affect the nervous system, resulting in peripheral neuropathy, demyelinating myelopathy, gait ataxia, depression, cognitive impairment, and dementia [7-9,20,21]. Although low plasma B-12 status has been associated with cognitive impairment and dementia in case-control studies, and researches have argued that vitamin B12 is somehow bound to cognition and to the implementation of active strategies to coordinate and do well in active problem solving, both cross-sectional and prospective cohort studies have shown mixed associations of serum vitamin B-12 concentration with specific measures of cognitive function [1,22].

A higher prevalence of lower serum vitamin B-12 levels have been found in subjects with dementias and in people with different cognitive impairments, as compared with controls. In contrast, other cross-sectional studies have failed to find this association. Furthermore, some intervention studies have shown the effectiveness of vitamin B12 supplementation in improving cognition in demented or cognitively impaired subjects [1,20]. We believe that, improvement in SMMSE scores of the patients in group 1 after the vitamin B-12 replacement therapy supports the importance of vitamin B-12 in cognitive and mental process. We administered Vitamin B-12 orally to the group 1 because enteral medications are easier to administer and are often preferred in the treatments [23].

Considering the exclusion criteria, we designed the study groups according to serum vitamin B-12 levels. Our results revealed that if an individual has no vitamin B-12 deficinecy (in our study, above 197 pg/ml), SMMSE scores and ERP studies would also be within normal ranges and be independent of serum vitamin B-12 levels. However, we also found that if an individual has low vitamin B-12 levels together with cognitive complaints, he or she would be more susceptible to have lower SMMSE scores and altered ERP parameters when compared to groups 2 and 3. It is well known that ERPs depend on various factors including stimulus relevance, task performed, patient psychological and affective state, underlying lesions of the nervous system, and the use of drug [24]. In ERP studies, P300 component reflects the mental processes such as recognition, categorization of stimuli, expectancy or short-term memory while there are many regions in the brain, especially in the temporal lobe, the parietal lobe and the hippocampus, which are thought to be responsible for its generation. The numerous clinical P300 studies, strongly suggest that this ERP component may be clinically useful as an index of cognitive function [7]. Morover one of the recent studies has shown that there is high incidence of reversible cognitive impairment and P300 abnormalities in B12 deficiency neurological syndromes [25]. In our study, improvements in P300, N200 and P200 amplitudes and latencies (recorded from both parietal and frontal sites) together with SMMSE scores after vitamin B-12 replacement therapy in group 1 suggests that there is a close relationship between the cognitive functions and vitamin B-12. Of these three potentials, P300 has given the most reliable results, however, N200 and P200 may also be useful in the assessment of the eficaccy of vitamin B-12 replacement therapy.

ERPs and SMMSE parameters were used to assess the influence of vitamin B-12 replacement therapy in patients with cognitive decline due to vitamin B-12 deficiency. Our study results suggest that improvement in P300, N200 and P200 amplitudes and latencies together with SMMSE scores are useful indicators in the assessment of vitamin B-12 replacement therapy in patients with impaired cognitive functions due to vitamin B-12 deficiency. Patients with cognitive impairment due to vitamin B-12 deficiency can be followed-up by using ERPs parameters (specifically P300) during the vitamin B-12 replacement therapy. Further and well-designed studies are needed to confirm our results.

Competing interests

The authors declare that they have no competing interests.

References

1. Malouf M, Grimley EJ, Areosa SA. Folic acid with or without vitamin B12 for cognition and dementia. Cochrane Database Syst Rev 2003;4:4514.
2. Vogel T, Dali-Youcef N, Kaltenbach G, Andrès E. Homocysteine, vitamin B12, folate and cognitive functions: a systematic and critical review of the literature. Int J Clin Pract 2009;63(7):1061-7.
3. Mankad K, Kullmann DM, Davagnanam I. Neurological manifestation of vitamin B12 deficiency. Am J Med 2010;123(6):e1-2.
4. Kotchoubey B. Event-related potentials, cognition, and behavior: a biological approach. Neurosci Biobehav Rev 2006;30(1):42-65.
5. Van der Ham IJ, van Strien JW, Oleksiak A, van Wezel RJ, Postma A. Temporal characteristics of working memory for spatial relations: An ERP study. Int J Psychophysiol 2010;77(2):83-94.
6. Verleger R. On the utility of P3 latency as an index of mental chronometry. Psychophysiology 1997;34(2):131-56.
7. Lai CL, Lin RT, Liou LM, Liu CK. The role of event-related potentials in cognitive decline in Alzheimer's disease. Clin Neurophysiol 2010;121(2):194-9.
8. Matsui H, Nishinaka K, Oda M, Kubori T, Udaka F. Auditory event-related potentials in Parkinson's disease: prominent correlation with attention. Parkinsonism Relat Disord 2007;13(7):394-8.

9. Bruder GE, Kroppmann CJ, Kayser J, Stewart JW, McGrath PJ, Tenke CE. Reduced brain responses to novel sounds in depression: P3 findings in a novelty oddball task. Psychiatry Res 2009;170(2-3):218-23.
10. Oishi M, Mochizuki Y. Improvement of P300 latency by treatment of vitamin B12 deficiency. Case report. J Clin Neurophysiol 1998;15(2):173-4.
11. Moretti R, Torre P, Antonello RM, Cazzato G, Bava A. Vitamin B12 - defect:what does it mean to cognition? Eur J Neurol 2001;8(8):731.
12. Varela-Moreiras G, Murphy MM, Scott JM. Cobalamin, folic acid, and homocysteine. Nutr Rev 2009;67(1):69-72.
13. Moretti R, Torre P, Antonello RM, Cazzato G. Is isolated vitamin B12 deficiency a sufficient causative factor of dementia? Eur J Neurol 2001;8(1):87-8.
14. Carmel R. Efficacy and safety of fortification and supplementation with vitamin B12: biochemical and physiological effects. Food Nutr Bull 2008;29(3):177-87.
15. Shane B. Folate and vitamin B12 metabolism: overview and interaction with riboflavin, vitamin B6, and polymorphisms. Food Nutr Bull 2008;29(1):5-19.
16. Cravens DD, Nashelsky J, Oh RC. Clinical inquiries. How do we evaluate a marginally low B12 level? J Fam Pract 2007;56(1):62-3.
17. Hvas AM, Nexo E. Diagnosis and treatment of vitamin B12 deficiency-an update. Haematologica 2006;91(11):1506-12.
18. Sumner AE, Chin MM, Abrahm JL, Berry GT, Gracely EJ, Allen RH, et al. Elevated methylmalonic acid and total homocystein levels shows high prevalence of vitamin B12 deficiency after gastric surgery. Ann Intern Med 1996;1240(5):469-76.
19. Snow CF. Laboratory diagnosis of vitamin B12 and folate deficiency: A guide for the primary care physician. Arch Intern Med 1999;159(12):1289-98.
20. Healton EB, Savage DG, Brust JC, Garrett TJ, Lindenbaum J. Neurologic aspects of cobalamin deficiency. Medicine 1991;70(4):229-45.
21. Blumenthal DT, Glenn MJ. Neurologic manifestations of hematologic disorders. Neurol Clin 2002;20(1):265-81.
22. Savage DG, Lindenbaum J, Stabler SP, Allen RH. Sensitivity of serum methylmalonic acid and total homocysteine determinations for diagnosisng cobalamin and folate deficiencies. Am J Med 1994;96(3):239-46.
23. Andrès E, Fothergill H, Mecili M. Efficacy of oral cobalamin (vitamin B12) therapy. Expert Opin Pharmacother 2010;11(2):249-56.
24. Hansenne M. The p300 cognitive event-related potential. II. Individual variability and clinical application in psychopathology. Neurophysiol Clin 2000;30(4):211-31.
25. Kalita J, Misra UK. Vitamin B12 deficiency neurological syndromes: correlation of clinical, MRI and cognitive evoked potential. J Neurol 2008;255(3):353-9.